PROJECT # 21 31 I 73

EMC - 21E - stored eggs 2 weeks

PROCEDURE FOR FIXATION OF EGG BLASTODERM (revised from procedure of 19 I 73)

1. Apply a teflon ring on top of the albumin layer surrounding the blastoderm
2. Fill the ring with 3% glutaraldehyde in 0.1M PO_4 buffer and drip the fixative solution around the outside of the ring.
3. Allow the fixative to remain in the ring for 1 - 1 1/2 hours before removing the blastoderm from the yoke. After the fixative has been in the ring for 15 min., inject fixative under the blastoderm at various positions around the outside of the ring. About 2 ml. of fixative is injected into each egg.
4. Cut around the ring with a Justrite #12 curved blade and lift the ring with the blastoderm attached free from the yoke.
5. Transfer the ring and blastoderm to fresh fixative in a 35X10 petri dish and allow to continue fixing for 1 hour. Ring side should be down.
6. Rinse 3 times in 0.1M PO_4 buffer for a total of 1 hour.
7. Fix in 1% Osmium in 0.1M PO_4 buffer for 1 hour at room temperature.
8. Dehydrate in acetone series 10%,30%,50%,70%,100% for 10 min. each.
9/ Epon embedding - epon 1:1 with acetone open overnite to evaporate.

   epon 100% 2 days at room temperature

   epon 80C

Each blastoderm was kept separate : EMC-21EI
EMC-21EI
EMC-21EIII
EMC-21EIV
EMC-21EV

EMC - 21 F - Fresh eggs - stored over night in osmium in refrigerator

EMC - 21 G - 4 week stored " " " "

EMC - 21 H - 6 week stored " 2 % osmium only "

EMC - 21 I - Fresh eggs (white) " " " "

EMC - 21 J - 8 week stored - 6 % glut-PO_4 2 hr in refig.

EMC - 21 K - 2 week stored(white) "

EMC - 21 L - 2 week stored(white) 6% glut - collidine buffer